porphyrin macrocycles. Metal organic materials (MOMs), which are porous frameworks constructed from molecular building blocks composed of metal-organic ligand clusters of specific geometry, offer an excellent platforms for the development of heme biomimetic catalytic systems with unprecedented catalytic diversity. This is due to the fact that the metalloporphyrins can be encapsulated into specifically designed cavities within the porous framework leaving larger cavities available to catalytic substrates. Here we report the synethsis and characterization of several members of this new class of biomimetic heme MOMs (MOMzymes) based upon the HKUST-1 topology. These systems contain an iron porphyrin encapsulated within small cavities within the HKUST-1 framework and exhibit heme-protein like peroxidase activity.

Emerging Single Molecule Techniques

2565-Pos Board B551
2-Aminopurine Single-Molecule Fluorescence
Elvin A. Alemán, David Rueda.
Base flipping is a local conformational change that results in the rotation of a nucleotide out of its helical structure. This is frequently observed in numerous nucleic acid structural rearrangements, such as in DNA-protein interactions and in different RNA enzymes. 2-Aminopurine (2AP) is a fluorescent nucleotide analog extensively used to probe local conformational changes in nucleic acids in bulk experiments, but no single molecule approaches have been developed to study these mechanisms using 2AP. One challenge towards this goal is that 2AP fluorescence is very sensitive to the interaction with neighboring nucleotides, and its emission spectrum partially overlaps with that of tryptophan. We have developed new click-chemistry-based surface immobilization approach that enables us to monitor, in real time base flipping in DNA.
We have characterized the fluorescence properties of single 2AP labelled DNAs. Our results show that nucleotides are very dynamic in a single- or double-stranded DNA and that bases can flip out of the helix, suggesting that the base flipping occurs frequently and in a much slower time scale that previously believe. This new assay can also be used to study other local conformational changes in nucleic acids at the single-molecule level by using 2AP as the base flipping probe.

2566-Pos Board B552
Convex Lens-Induced Confinement for Imaging Single Molecules
Sabrina R. Leslie, Alex Fields, Adam Cohen.
Fluorescence imaging is used to study the dynamics of a wide range of single molecules in solution or attached to a surface. Two key challenges in this pursuit are to image immobilized single molecules in the presence of a high level of fluorescent background, and to image freely diffusing single molecules for long times. We present a simple modification to a wide-field fluorescence microscope that addresses both challenges and dramatically improves single-molecule imaging. The Convex Lens Induced Confinement (CLIC) system restricts molecules to a wedge-shaped gap of nanoscale depth. The shallow depth of the imaging volume leads to up to 20-fold greater rejection of background fluorescence than is achieved with total internal reflection fluorescence (TIRF) imaging. The elimination of out-of-plane diffusion leads to approximately 10,000-fold longer diffusion-limited observation time per molecule than is achieved with confocal detection in free solution. The restriction of freely diffusing molecules to gaps whose depth is greater than the molecular diameter provides a simple measure of molecular size. The CLIC system may be implemented using a minor modification to a standard flow cell, and does not require any nanofabrication, nor any custom optics, electronics, or computer control.
We demonstrate the advantageous properties of CLIC measurements by imaging singly labeled surface-immobilized DNA molecules in the presence of a high concentration of free dye (up to 2 micromolar); by counting the transmembrane proteins in freely diffusing lipid vesicles; and by measuring the sizes of freely diffusing proteins and DNA with diameters ranging from 3nm to 200 nm. Further, we apply CLIC to probe weak intermolecular interactions in a range of systems.

2567-Pos Board B553
Molecular Interactions on the Plasma Membrane Studied by STED Fluorescence Microscopy
Christian Eggeling, Veronika Mueller, Steffen J. Sahl, Marcel Leutenegger, Andreas Schönle, Stefan W. Hell.
The direct and non-invasive observation of a whole range of cellular functionalities is impeded by the resolution limit of >200nm of a conventional far-field optical microscope. Prominent examples are molecular interactions on the plasma membrane such as the integration into lipid nanodomains ('rafts'), which are considered to play a functional part in a whole range of membrane-associated processes. We report the detection of single diffusing lipid and protein molecules in nanosized areas in the plasma membrane of living cells using the superior spatial resolution of stimulated emission depletion (STED) far-field nanoscopy or of fast single-molecule tracking. By combining a (tunable) resolution of down to 30 nm with tools such as fluorescence correlation spectroscopy (FCS) or by spatio-temporally following the movement of single lipids, we obtain new details of molecular membrane dynamics. For example, we reveal transient (~ 10 ms) trapping of certain sphingolipids on the nanoscale in cholesterol-mediated molecular complexes. However, distinct differences show up between different lipids and molecules. Molecules such as certain transmembrane proteins show both trapping and a kind of hopping diffusion. The novel observations may highlight new details on lipid-protein interactions and their role in membrane bioactivity.

2568-Pos Board B554
Mechanochemical Cycle of Hsp90
Christoph Ratzke, Thorsten Hugel.
The molecular chaperone Heat-Shock-Protein 90 (Hsp90) is an important and abundant protein in eukaryotic cells, essential for the activation of a large set of signal transduction and key regulatory proteins. Its function is dependent on an exceptionally slow ATP hydrolysis of about one per minute and the functional Hsp90 dimer performs large conformational rearrangements, both at its N-terminus[1,2] and its C-terminus[3], whereas a communication between N- and C-terminal domains could be found[3]. In addition, we extended our setup to three color FRET and show first results on the coordination between nucleotide binding and the conformational dynamics.
[1] Ali, M.M., Roe, S.M., Vaughan, C.K., Meyer, P., Panaretou, B., Piper, P.W., Prodromou, C., and Pearl, L.H. Nature, *440*, 1013 (2006).
[2] M. Mickler, M. Hessling, C. Ratzke, J. Buchner, T. Hugel, NSMB, 16, 281 (2009)
[3] C. Ratzke, M. Mickler, B. Hellenkamp, J. Buchner, T. Hugel, PNAS, 107, 16101 (2010)

2569-Pos Board B555
Submicro to Millisecond Conformational Transitions of Bacteriophage T4 Lysozyme with Ångström Accuracy using Multiparameter Fluorescence Detection
Hugo Sanabria, Dmytro Rodnin, Stanislav Kalinin, Suren Felekyan, Mark R. Fleissner, Wayne Hubbell, Claus A.M. Seidel.
We use single molecule FRET and the completeness of Multiparameter Fluorescence Detection to study protein flexibility and dye rigidity from submicro- to millisecond timescales in native and non-native conditions of the bacteriophage T4 Lysozyme (T4L).
T4L contains 164-residues and consists of two domains connected by a long alpha helix. The enzyme cleaves the glycosidic bond between N-acetylmuramic acid and N-acetylglucosamine of bacterial cell wall saccharides. From crystalographic and EPR measurement the native enzyme is believed to be in dynamic exchange between various "open" conformations. Most likely a set of hinges is responsible for the dynamic motion of the enzyme. However, the time scale of these hinges is still unknown. To accurately determine protein conformations from single molecule FRET, one needs to consider the dynamic behavior of donor and acceptor fluorophores. To test protein and dye mobility, we used single and double labeled T4L mutants with various fluorophore linkers using site specific mutants. The orthogonal system contains a ketone handle in the N-terminal domain for reaction with a hydroxylamine or hydrazide fluorophore, or a thiol group in the C-terminal domain for reaction with a thiol-specific fluorophore.
Furthermore, the double mutant is ideal for site-specific attachment of the donor and acceptor fluorophores. Beside studies on native conformational dynamics we look into non native conformational transitions using chemical denaturants. In addition, we complement our experimental work by modelling the accesible volume (AV) required for each fluorophore using steric interaction with known crystalographic data. The AV model considers all available locations where the dye could be located without causing sterical clashes. This tool in combination with single molecule FRET allows us to quantitatively determine corresponding structural distances from experimental data.

2570-Pos Board B556
Single Molecule FRET Studies of Protein Conformational Landscapes: 3 Prototypic Examples for the Relation Between Conformational Dynamics and Function
Markus Richert, Dymitro Rodnin, Carola S. Hengstenberg, Thomas Peulen, Alessandro Valeri, Paul J. Rothwell, Pohl Millon, Marina Rodnina, Christian Herrmann, Claus A.M. Seidel.
Since the major structural biology method, X-ray crystallography, is limited to resolving fixed homogeneous conformations, techniques like single molecule FRET are becoming more important to determine dynamic behaviour of proteins. In comparing three different single molecule FRET studies of prototypic proteins we show that their structural aspects and dynamic behaviour are closely related to function. Initiation factor 3 (IF3) plays an important role in protein synthesis since it is an activating ligand for the correct assembly of a ribosome. It could be shown, that